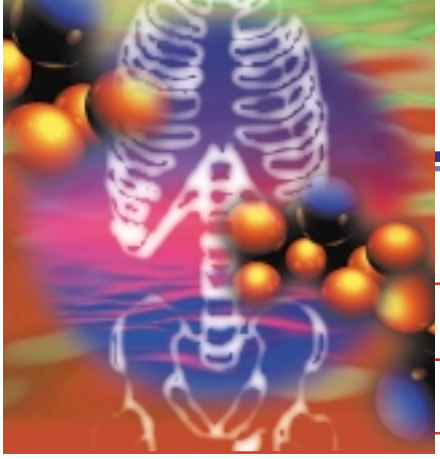


Shimadzu Developments in Biotechnology

μ-TAS (micro-Total Analysis System) Paves the Way for 21st Century Biotechnology

Higher Sensitivity and Greater Speed Lab-on-a-Chip Technology Accelerates the Development of Genomics and Proteomics

Developments in biotechnology, particularly in genetic engineering and protein engineering, are likely to dramatically alter society in the 21st Century. Genetic engineering should contribute to solving the world's food problems by creating high-yield wheat able to withstand severe environments. It should also lead to revolutionary new drug treatments for cancer and other diseases resulting from genetic abnormalities and more effective diagnostic drugs and drug treatments that accommodate the slight genetic variations between individuals (so called tailor-made treatments). Gene recombination may result in the dramatic development of new waste disposal systems using micro-organisms able to break down plastics at astonishing rates.

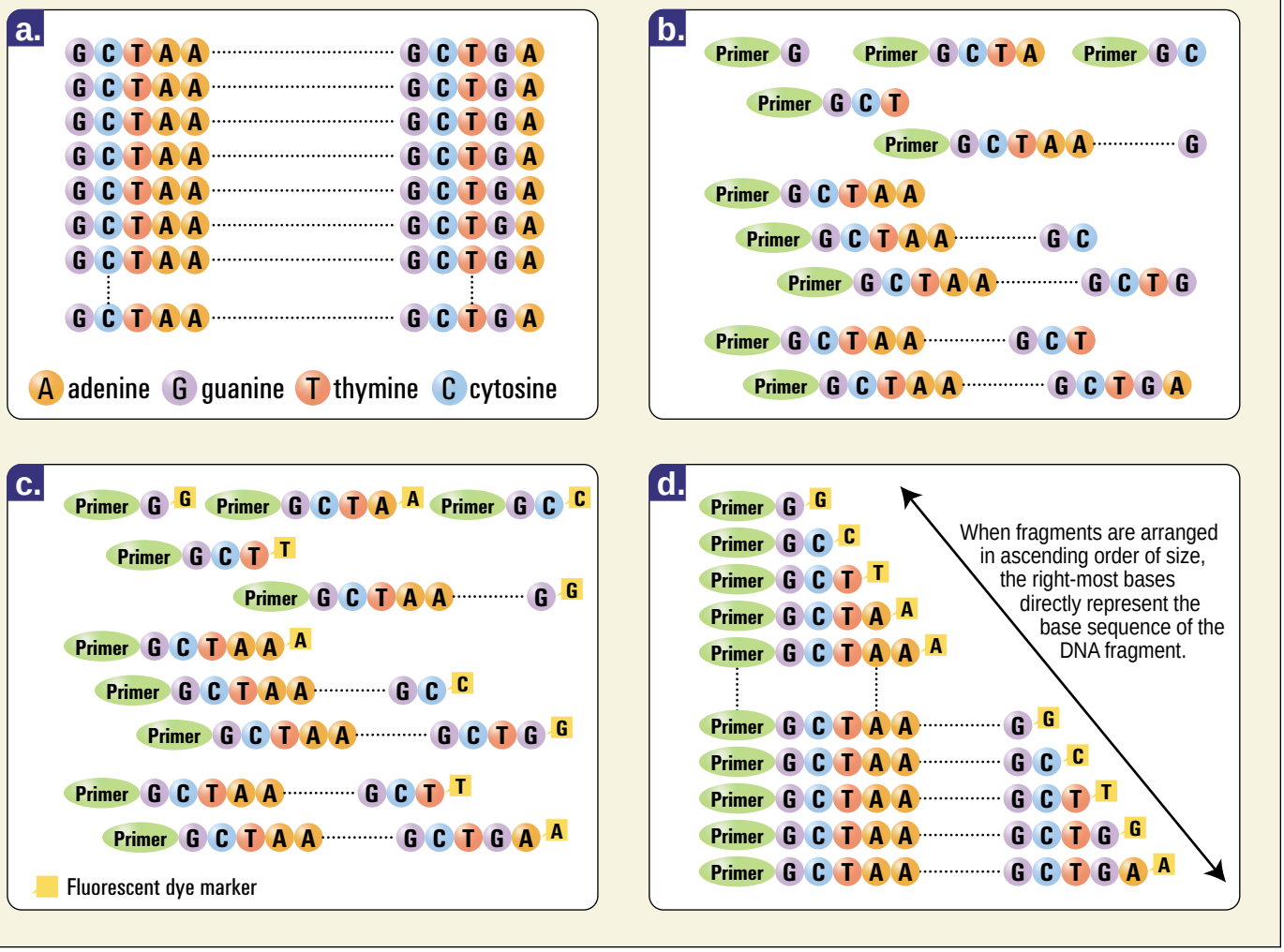
High-performance Sequencers Contribute to Sequencing the Genome

However, even if these broad projections are correct, the actual steps to achieving them are difficult and many technical problems remain to be tackled. One topic that is becoming increasingly important is improving the performance of the equipment used for research and development, including automatic DNA sequencers.

The last year of the 20th century saw the writing of a rough draft of the human genome that contains all human genetic information, a monumental achievement comparable to the Apollo moon landings. Most of the human genome structure, containing some 3 billion base pairs of the four bases - adenine, guanine, thymine, and cytosine - was revealed. While this achievement was based on the hard work of the scientists involved, the result also depended on the rapid performance improvements of the equipment supporting the research. Increased sequencer performance was especially significant. Due to improvements in sequencer performance, capacity to analyze human genome data improved exponentially toward the end of the 90s. In fact, it increased so much so that half of the genome was analyzed after the start of the year 2000.

But an equivalent level of performance improvement is still expected in the future because sequencing the human genome is not the final goal, but rather just the beginning. Which parts of the 3 billion base pairs provide which genetic functions? What physiological changes arise due to a single changed base pair? What are the genetic structures of fauna and flora, or micro-organisms, both harmful and beneficial to humans?

Principle of DNA Sequencing Analysis



How do the genes function? Most of these questions remain to be solved in the future. The genomes of other living things waiting to be analyzed are no smaller than the human genome. The human genome contains 3 billion base pairs; bird genomes from several hundred million to several billion base pairs; and fish genomes from several hundred million to ten billion base pairs. Amazingly, some amphibian and plant genomes contain several tens of billions of base pairs!

DNA Sequencing a Time and Labor Intensive Task

In addition, sequencing work using currently established DNA sequence analysis principles requires a lot of time and labor. The procedure and principle of this analysis is outlined below.

Firstly, chromosomes, which are part of the genome, are extracted from cells. Despite being small, chromosomes comprise several tens of millions of bases, so they are further broken down into DNA fragments containing approximately 500 to 1000 bases. Next, a method called the polymerase chain reaction uses repeated heating and cooling to cause repeated disassociation and re-association of duplex DNA, thereby creating many copies of the fragments. This stage simply creates large numbers of copies of the four bases [adenine (A), guanine (G), thymine (T), and cytosine (C)] in the same order, although the actual sequence is still unknown. (See Figure a.)

From these fragments are then

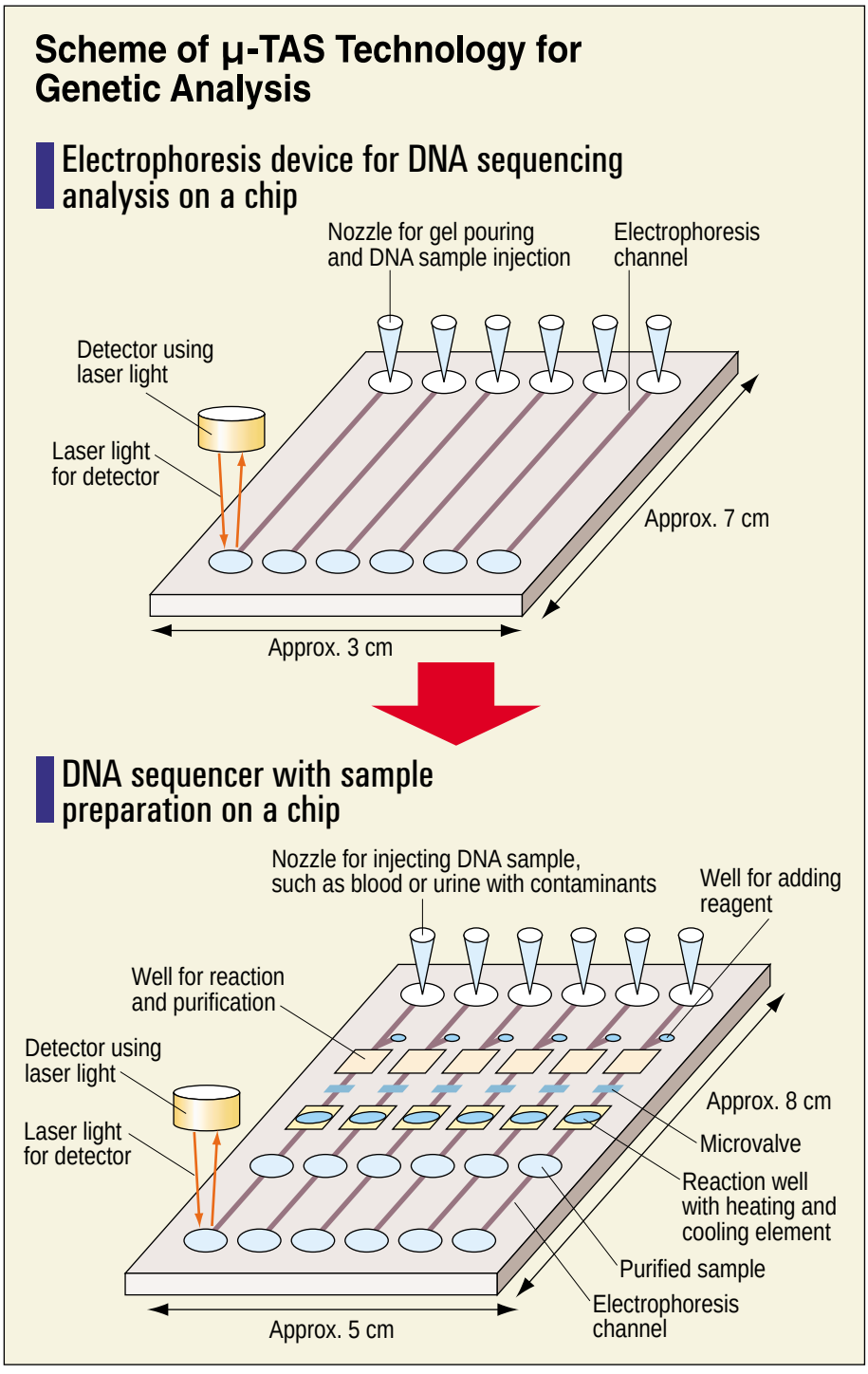
made DNA fragments of various lengths (that is, various numbers of bases) with a special anchor fragment called a primer attached to one end and one of the bases A, G, T, or C at the other end. Although each fragment contains various numbers of bases, if the original fragment contained 500 bases, for example, the resulting fragment will not exceed this number.

In principle, the primer may be attached to one base or up to 500 bases, such that large numbers of DNA fragments are created, each differing in length by one base. The sample at this time is a mixture of large and small fragments with unknown DNA sequences. (See Figure b). Next, a fluorescent dye is used to distinguish between DNA fragments with A, G, T, or C at the non-primer end. (See Figure c). The actions up to this stage are basically sample preparation.

Exploting the Difference of Migration Speed Due to the Difference of a Single Base

The DNA fragments of different lengths following the sample preparation are identified according to length using a method called capillary electrophoresis. Capillary electrophoresis involves filling a 100 μm diameter, 50 cm long glass tube with a gel matrix and applying a positive and negative voltage to opposite ends. The sample of pre-treated DNA fragments is inserted at the negative voltage end. As DNA is originally negatively charged, the sample begins to move toward the positive end. However, the gel matrix hinders the movement and a phenomenon occurs whereby the larger the fragment (that is, the more bases that are connected to form the fragment), the more slowly it proceeds. Conversely, shorter fragments reach the positive end more quickly.

The relationship between the migration speed and the size of a fragment is very strict. The difference of just a single base slightly affects the time taken for the fragment to arrive. Laser light at the



positive end of the capillary detects the four types of fluorescent markers, and arranging the fragments in the sequence they arrived directly indicates the A, G, T, C base sequence. The DNA fragments of different lengths can be considered to be arranged in sequence, each differing by one base. (See Figure d). As the base (A, G, T, or C) at the non-primer end of each fragment is known, arranging the fragments sequentially in ascending order of length reveals the base sequence of the DNA fragment. A sequencer is an instrument that automatically conducts electrophoresis under computer control.

It normally takes about three hours to analyze one sample of DNA fragments by electrophoresis. Analyzing the 3 billion DNA bases 500 bases at a time requires a total of 6 million samples. That would take a lot of work. But no better method of DNA sequencing analysis has been established to date. Efficiency can be enhanced by increasing the number of glass tubes in the DNA sequencer to analyze many samples simultaneously, but even the most sophisticated sequencers in the world (Shimadzu RISA-384) can currently analyse only 384 samples at one time.

All Steps Including Preparation on a Single Chip

The technologies expected to break these barriers are the μ-TAS (micro-Total Analysis System) and lab-on-a-chip technology. This is a biological or chemical analysis system, such as an electrophoresis device, integrated onto a quartz substrate several centimeters square much like a semiconductor integrated circuit. For example, 50 μm wide, 30 μm deep, and 5 cm long channels might replace the 100 μm diameter, 50 cm long glass tubes. The current aim is to use semiconductor chip manufacturing technology to create systems with devices such as electrodes, sensors, ultrasmall pumps and valves formed at the same time as the flow channels.

This ultra-compact device using μ-TAS has the dual benefits of consuming only microscopic amounts of sample and expensive reagents for the analysis and of reducing the analysis time. For example, an electrophoresis device on a chip such as the one described above could complete the analysis of a DNA fragment sample in about 15 minutes. Consequently, processing performance equivalent to the top full-size sequencers currently on the market could be achieved from a system with 32 channels on a chip. It is not difficult to produce 32 channels 50 μm wide and 30 μm deep on a chip.

A chip-based system that even handles the DNA sample preparation required for DNA sequencing analysis should be achieved in the near future. It is also possible to create an automated system to conduct DNA sequencing analysis on living tissue, such as a blood vessel, inserted into a particular well in the chip. Systems to diagnose genetic disease are also feasible. Of course, miniaturization of the controlling computer and laser detector instruments is also required, and the aim of μ-TAS is to create a total system that includes these.

After the genome DNA sequence has been decoded and gene functions identified, the next topic facing biotechnology is proteomics - research into the structures and functions of the many proteins created by genes.

μ-TAS technology is expected to also significantly contribute to the development of proteomics research.

Microchip Electrophresis